MATCHING SECRETION CAPACITY VIA TRANSLATIONAL CONTROL

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Inducible gene expression systems commonly employed in microbial hosts are mostly composed of molecular components derived from the lactose (lac), arabinose (ara), rhamnose (rha) and tetracycline (tet) operons. These transcription-level control systems have been widely employed in research and industrial-scale protein production, however they are known to exhibit important limitations. These include; all-or-none (digital) expression profiles, stochastic transcriptional bursting, and heterogeneous expression responses at sub-maximal induction conditions. A new paradigm in genetic regulation emerged with the discovery of genetic regulatory elements within the 5'UTR of bacterial mRNA [1]. Upon binding to a specific metabolite, these so-called riboswitches change conformation, permitting differential gene regulation to occur. To allow us to utilize this alternative mechanism of genetic regulation we developed and characterized a novel recombinant expression system, termed *RiboTite* [2]. The system operates at both the transcriptional and translational level, using standard inducible promoters and translational-ON riboswitches respectively, collectively providing a multi-layered modular genetic circuit controlling both bacteriophage T7 RNA polymerase and recombinant gene(s) of interest [3]. The precise cellular-level tunable expression control afforded by this system offers a number of potential applications in terms of matching cellular expression rate to host synthetic and processing capacity. Here we will report one such application, where we used the *RiboTite* system to avoid the overload of secYEG translocon in *E. coli*, permitting expression/secretion attenuation of recombinant proteins into periplasmic space (Figure 1). Utilizing a library of different signal peptides that target the recombinant protein to secYEG, either via the post-translational (SecB/A) or the co-translational (SRP) pathway, we have demonstrated successful attenuation of recombinant protein reaching the periplasm. Finally under fed-batch fermentation conditions the system has been demonstrated to avoid the overload of the host secretion machinery and produce scFv antibody fragments at industrially relevant titers.

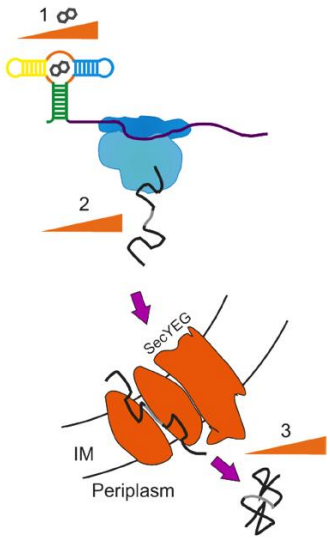

Figure 1 – Matching expression demand to secretion capacity

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[2] Morra, R et al. Dual transcriptional-translational cascade permits cellular level tuneable expression control Nucleic Acids Research 2016 18 (e21)
[3] Muhamadali, H, et al. Metabolomic analysis of riboswitch containing E. coli recombinant expression system Molecular BioSystems 2016 12 350